

Composition of Cigarette Smoke From High- and Low-Nitrate Burley Tobacco¹

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Introduction

Recent reports have indicated that the treatment of cigarette tobacco with either sodium or potassium nitrate results in a selective reduction of certain smoke constituents (2,4). In one of these reports the addition of 8.3% sodium nitrate resulted in a significant lowering of total particulate matter, nicotine, benzo[*a*]pyrene and phenol in the smoke from treated cigarettes as compared with standard (untreated) cigarettes (2). When painted on the backs of mice, the smoke from the treated cigarettes was also less toxic and resulted in a lower tumor yield than that from the standard cigarettes. The action of sodium nitrate in reducing these substances in smoke was attributed to its contributing oxygen, which facilitated a more complete combustion of the tobacco. It was also thought that the nitric oxide which formed when the nitrate was heated could function as a free-radical scavenger and thereby inhibit the formation of polynuclear aromatic hydrocarbons.

Cigarettes manufactured from Burley tobacco grown under two

different rates of nitrogen fertilization were available to us. The analysis of these two lots of tobacco showed a 17-fold difference in naturally occurring nitrate. It therefore was considered worthwhile to determine whether those smoke components which were altered by addition of sodium nitrate to tobacco also would be found in significantly different amounts in the smoke from these two tobaccos.

Two lots of Burley #37 tobacco were grown on adjacent plots at the Tobacco Experiment Station, Greenville, Tenn. The low-nitrogen plot received fertilizer in the form of 20% superphosphate and 50% potassium sulfate but no nitrogen. The high-nitrogen plot received 5-10-5 fertilizer plus additional ammonium nitrate and potassium sulfate constituting a total of 300 lb nitrogen per acre. This difference in nitrogen fertilization was reflected in a difference in the nitrate content of the leaf. The tobacco receiving no nitrogen contained 0.098% nitrate while that fertilized with nitrogen contained 1.66% nitrate. Nitrogen determinations on these tobaccos were made using the method described by Broadus (5). Both groups of cigarettes were manufactured on commercial machines, were 85 mm in length, and were unfiltered. Prior to smoking they were conditioned at 25°C and 60% relative humidity.

Smoking Conditions and Collection of Tar

Twenty cigarettes were smoked for each set of determinations. These were smoked to an approximate 23 mm butt length using a

CSM-10 Smoking Machine.³ A 35 ml puff of 2 seconds duration was taken once a minute on each cigarette. The smoke was collected at room temperature in 50 ml of vigorously stirred cyclohexane contained in a 250 ml flask. Addition of the smoke beneath the surface of the cyclohexane was avoided to eliminate a possible pressure drop. Collection of smoke in this manner was very efficient. During the smoking of groups of 20 cigarettes no significant amount of particulate matter deposited on a Cambridge filter which was placed behind the collection flask in the smoking train.

Fractionation of Smoke (Figure 1)

After the smoke was collected, the cyclohexane solution was transferred to a separatory funnel. The collection flask, stirrer, and inlet tube were rinsed with alternate portions of 2x5 ml of 0.5 N sodium hydroxide and 3x5 ml of cyclohexane. After removing the aqueous layer, the cyclohexane solution was extracted with 3x5 ml of 0.5 N sodium hydroxide followed by 1x5 ml saturated NaCl sodium chloride. The extracts were combined and backwashed with 2x5 ml of cyclohexane. This base extract (A) was used to determine phenols. The combined cyclohexane solution was extracted with 5x5 ml of 0.5 N hydrochloric acid followed by 1x5 ml of saturated sodium chloride. The extracts were combined and backwashed with 2x5 ml of cyclohexane which was added to the main cyclohexane solution. This acid extract (B) was used for the determination of nicotine.

The cyclohexane solution was next extracted with 5 ml of methanol-

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³ Mention of specific commercial products does not constitute endorsement by the United States Department of Agriculture or the Kentucky Agricultural Experiment Station.

water (4:1). After separation the methanol-water solution was back-washed with 2x10 ml and 4x5 ml of cyclohexane which was added to the main cyclohexane solution. The cyclohexane solution was next extracted with 2x10 ml and 6x5 ml of nitromethane. These nitromethane extracts were combined and the solution concentrated to dryness using a rotary evaporator (bath temp. 45°C; press. 30 mm). The residue (C) was used for the determination of benzo[*a*]pyrene.

Determination of Phenols

The base extract (A) was steam distilled. Approximately 500 ml of distillate was collected and discarded. The contents of the distillation flask were allowed to cool and then acidified with 50 ml of 20% sulfuric acid and again steam distilled. The distillate (180 ml) was acidified and extracted with 6x50 ml of purified ether. The ether extracts were combined and extracted with 2x5 ml of saturated sodium bicarbonate solution. The ether solution was dried over anhydrous sodium sulfate and concentrated to less than 1 ml by distillation through a 13 cm Vigreux column at atmospheric pressure. This concentrated ether solution was transferred to a 1 ml volumetric flask and diluted to volume. This solution was used to determine phenols by gas chromatography, using a Barber-Colman Selecta 5000 system with a model 5043 electrometer and a flame ionization detector. A 2m x 1/8 in. s.s. column packed with 100-120 mesh Porapak Q was used.

The operating parameters were: nitrogen carrier gas flow 25 ml/min; air and hydrogen gas flows, 300 ml and 27 ml/min respectively; column, injector and detector temperatures, 230°, 250°, and 270°C, respectively. The electrometer settings were: sensitivity, 10⁻¹⁰ amps and attenuation, 2. An Infotronics Digital Readout System, Model CRS-11 HSB, was used to determine peak areas. The settings on the integrator were: tracking rate, 3 up — 10 down; threshold level, 0.05; trip, 4; slope sensitivity, 1; and filter frequency, 1.

The concentration of phenol, *o*-cresol, and *m*- and/or *p*-cresol was determined from the ratio of their individual peak areas to the area of *o*-chlorophenol which served as an internal standard. An aliquot of a standard solution containing 2 mg of *o*-chlorophenol was added to the cyclohexane solution immediately after the smoke was collected. The relative response and retention time of the phenols to *o*-chlorophenol

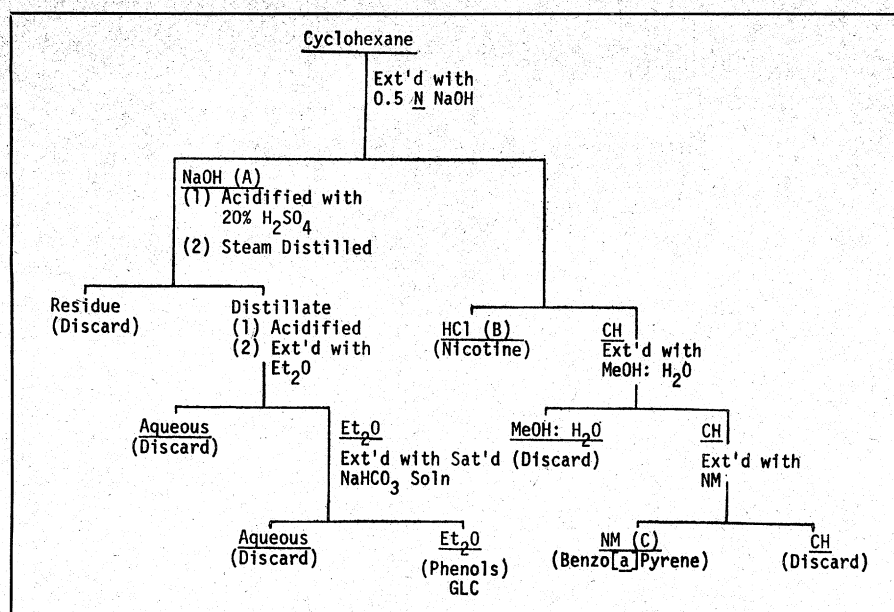


Figure 1. Fractionation of cigarette smoke.

were determined from a standard solution containing phenol, *o*-, *m*-, *p*-cresol and *o*-chlorophenol.

Determination of Nicotine

This procedure is a modification of that described by Ogg (3) for the determination of nicotine in smoke particulate matter collected on a Cambridge filter. The acid extract (B) and 10 ml of 0.5 N hydrochloric acid were steam distilled until 300-400 ml of distillate was collected, which was discarded. After the acidic solution in the distillation flask had been allowed to cool, 30 ml of a 30% sodium hydroxide solution, saturated with sodium chloride, was added. The resulting solution was then steam distilled until approximately 400 ml of distillate was collected. In this second distillation the distillate was collected under the surface of 50 ml of 0.5 N hydrochloric acid contained in a 500 ml volumetric flask. After the volume of the distillate was adjusted to 500 ml with distilled water, a 25 ml aliquot was diluted to 100 ml with 0.05 N hydrochloric acid. The absorbance of this solution was read at 236 mμ, 259 mμ and 282 mμ. Milligrams of nicotine per cigarette, N, was calculated using the formula:

$$N = \frac{A_{259} - (A_{236} + A_{282})/2}{0.3213 \times b}, \text{ where}$$

A_{259} , A_{236} and A_{282} are the absorbances at the indicated wavelengths and b is the pathlength of the cell in cm.

Determination of Benzo[*a*]pyrene

The residue (C) from the nitro-

methane-soluble fraction was dissolved in approximately 20 ml of benzene, placed on a 2.5 cm (i.d.) column containing 5 grams of silicic acid and the column was eluted with 250 ml of benzene which was collected in a single portion. This procedure removed the bulk of the polar chromophoric material. The eluant which contained the benzo[*a*]pyrene and other aromatic hydrocarbons was concentrated to dryness using a rotary evaporator (bath temp. 45°C; press. 30 mm). The weight of this residue is reported as the "nitromethane fraction." After the residue was weighed it was taken up in approximately 0.1 ml cyclohexane and applied as a series of spots, forming a 12 cm streak, to a 20x20 cm alumina thin-layer plate. Reference spots of authentic benzo[*a*]pyrene were placed 1.5 cm from each end of the streak. After the plate was developed in ethyl ether-pentane (1:9), the band containing benzo[*a*]pyrene was located by examining the plate under UV light and was transferred to a 15 ml centrifuge tube. The benzo[*a*]pyrene was desorbed with 4x4 ml of methanol. The four solutions were combined and concentrated to approximately 0.1 ml and chromatogrammed on a 20% acetylated cellulose thin-layer plate. After the plate was developed in ethanol-toluene-water (17:4:1), the benzo[*a*]pyrene band was transferred to a 15 ml centrifuge tube and recovered by desorbing with 4x4 ml of methanol. All methanol solutions were pooled, concentrated to approximately 0.3 ml, and filtered through a small cotton plug into a volumetric flask. After dilution to

Table 1. Physical Data on Cigarettes*

	Low Nitrate	High Nitrate	Commercial
Avg. wt./cig. (g)	0.83	0.96	1.17
Percent moisture	11.8	11.4	12.1
No. puffs/20 cig.	134	157	218
Avg. burn temp. (°C)	701	712	738

* Average of at least five determinations.

Table 2. Analyses of Cigarette Smoke*

	Low Nitrate	High Nitrate	Commercial
	<i>Per Cigarette</i>		
Wet TPM (mg)	21.5	28.4	29.8
Dry TPM (mg)	19.5	25.7	27.0
Nicotine (mg)	1.26	3.97	1.40
Nitromethane Fraction (mg)**	6.0	5.6	5.6
Benzo[a]Pyrene (ng)	7.4	7.2	11.5
Phenol (μg)	41	57	83
<i>o</i> -Cresol (μg)	8	9	15
<i>m</i> -, <i>p</i> -Cresol (μg)	20	16	35
Total phenols (μg)**	69	82	133
	<i>Per 100 ml Smoke</i>		
Dry TPM (mg)	9.6	10.1	7.8
Nicotine (mg)	0.53	1.45	0.37
Nitromethane Fraction (mg)**	2.6	2.2	1.5
Benzo[a]Pyrene (ng)	3.2	2.6	3.0
Phenol (μg)	18	21	22
<i>o</i> -Cresol (μg)	3	3	4
<i>m</i> -, <i>p</i> -Cresol (μg)	9	6	9
Total phenols (μg)**	30	30	35

** See text

* All values are averages of at least two determinations

and LN) are summarized in Table 1. For comparison, data obtained from an unfiltered, 85 mm, commercial cigarette are also presented. These data show that cigarettes made from the HN tobacco weighed more and burned slower than those made from the LN tobacco. The greater weight and slower burn rate of the blended cigarette compared with those of the Burley cigarettes are also evident. As pointed out by Touey and Mumpower (6), the use of thermocouple wire of a diameter greater than 0.002 inch results in low burn temperature measurements. Since the thermocouple used in this work was 0.012 inch in diameter the burn temperature values given in Table 1 are not absolute. These are reported only for comparative purposes. The difference in burn temperature between the HN and LN cigarettes is not thought to be significant.

Data on the levels of certain selected constituents in the smoke from these cigarettes are presented in Table 2. Values are shown on a "per cigarette" as well as a "per 100 ml of smoke" basis. Because of the difference in the weight of the cigarettes and the total number of puffs required to reach approximately equal butt lengths, data based on equal volumes of smoke are considered to be more meaningful for comparison.

On the basis of 100 ml of smoke, the HN cigarettes yielded slightly higher TPM than the LN cigarettes. TPM values for the commercial cigarettes were much lower than those for either of the Burley cigarettes when converted to mg/100 ml of smoke. As a result of the higher rate of nitrogen fertilization, the nicotine content of the HN tobacco was 5.40% whereas that of the LN tobacco was 2.84%. This gives a ratio of 1.9 for the nicotine content of these two tobacco types. Since the ratio of the nicotine contents in the smoke from both of these tobaccos was even higher (2.7), it is therefore apparent that a high level of naturally occurring nitrate in tobacco leaf is not significant in reducing the nicotine content of the smoke.

This is contrary to results obtained by the addition of sodium nitrate to tobacco (2,4).

Weights of the nitromethane-soluble fraction of the smoke are also given in Table 2. Because benzo[a]pyrene and other polynuclear aromatic hydrocarbons are contained in this fraction it was thought worthwhile to see if a relationship is indicated between the

2 or 5 ml the fluorescence spectrum of the solution was obtained, using an Aminco-Bowman Spectrofluorometer set at an excitation wavelength of 380 mμ. The fluorescence maximum at 405 mμ was read and the concentration of benzo[a]pyrene was determined with a standard curve.

Determination of Total Particulate Matter

Total particulate matter was determined from five cigarettes essentially as described by Ogg (3). The cigarettes were smoked to a 23 mm rather than a 30 mm butt length and the TPM is reported on both a "wet" and "dry" basis.

Determination of "Burn Temperature"

Temperature measurements were made using a 0.012 inch diameter Pt/Pt-13% Rh thermocouple connected in series with a reference

junction (0°C) to a potentiometer-type recorder. The thermocouple was inserted approximately 25 mm into the free end of the cigarette which was held in a Cambridge filter holder. The burn temperature was recorded as the point of maximum recorder response as the combustion zone passed over the thermocouple junction. For each determination 10 cigarettes were smoked, using a standard smoking cycle, and the values averaged.

Determination of Moisture

Water on both the Cambridge filters and in the tobacco was determined by means of a gas chromatographic procedure which will be described in a subsequent publication.

Results and Discussion

Physical data for the high and low nitrate Burley cigarettes (HN

weight of the nitromethane residue and the levels of benzo[*a*]pyrene in the smoke. The data obtained for all three cigarettes indicated, however, that there is no relationship between these values. This has also been shown to be the case with other experimental cigarettes analyzed in this laboratory. For the Burley cigarettes, comparison of the benzo[*a*]pyrene levels per 100 ml of smoke indicates that the benzo[*a*]pyrene content is slightly less in the smoke from the high nitrate tobacco.

Although no difference was found in the levels of total phenol (sum of phenol and cresols) in the smoke from the Burley cigarettes, a difference is indicated in the amounts of certain individual phenols. The phenol content of the smoke from the HN cigarettes has been shown to be higher and the levels of *m*- and *p*-cresols to be lower than that found in the smoke from the LN Burley cigarettes.

Although the level of constituents is lower in the smoke from the HN tobacco, the magnitude of this decrease is not very great in comparison with the results obtained by the addition of 8.3% sodium nitrate to tobacco. This is undoubtedly because, unlike the case in which 8.3% sodium nitrate was added to the tobacco, the level of naturally occur-

ring nitrate in the high nitrate tobacco was equivalent to only 2.28% sodium nitrate.

Summary

Levels of certain chemical constituents were determined in the smoke of cigarettes made from two lots of Burley tobacco containing a 17-fold difference in naturally occurring nitrate. On the basis of equal volumes of smoke, the high nitrate cigarettes yielded considerably more nicotine than the low nitrate cigarettes. The levels of other smoke constituents, however, were not found to differ greatly for the two cigarettes. Slightly lower levels of benzo[*a*]pyrene and *m*- and *p*-cresols were found in the smoke from the high nitrate cigarettes. The smoke from the low nitrate cigarettes was slightly lower in TPM and phenol. However, no difference in total phenols was found in the smoke from these cigarettes. For comparison, levels of these constituents in the smoke of commercial cigarettes were also presented.

Acknowledgment

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Literature Cited

1. Broadus, G. M., J. E. York and J. M. Moseley. Factors affecting the levels of nitrate nitrogen in cured tobacco leaves. *Tobacco Sci.*, 9:149-157, 1965.
2. Hoffmann, D. and E. L. Wynder. The reduction of the tumorigenicity of cigarette smoke condensate by addition of sodium nitrate to tobacco. *Cancer Res.*, 27: 172-174, 1967.
3. Ogg, C. L., Determination of particulate matter and alkaloids (as nicotine) in cigarette smoke. *J. Assoc. Offic. Agr. Chemists*, 47:356-362, 1964.
4. Rathkamp, G., D. Hoffmann and E. L. Wynder. Experiments on the reduction of polynuclear aromatic hydrocarbons in cigarette smoke. Program Abstract, 20th Tobacco Chemists' Research Conference, Winston-Salem, N. C., November 1-3, 1966, p. 23.
5. Sadler, W. W., R. R. Chesson and A. W. Schoenbaum, Automated procedure for determining the nicotine content of steam distillates. *Tobacco Sci.*, 4:208-212, 1960.
6. Touey, G. P. and R. C. Mumpower II, Measurement of the combustion-zone temperature of cigarettes. *Tobacco Sci.*, 1:33-37, 1957.